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(57) [Abstract]

[Purpose] The present invention relates to a method for controlling the blight of cultivated plants caused by microorganisms.

[Construction] The present invention relates to a method for controlling plant blight in which the plants are treated with an aqueous micelle solution or an aqueous dispersion of a mixture of a lysophospholipid and an unsaturated fatty acid and/or medium-chain fatty acid or a mixture of a lysophospholipid, an unsaturated fatty acid and/or medium-chain fatty acid, oil and fat.

[Claims]

[Claim 1] A method for controlling plant blight, wherein an aqueous solution comprising a lysophospholipid and fatty acid mixture is applied to plants.

[Claim 2] The method described in claim 1, wherein the lysophospholipid to fatty acid mole ratio is 0.5-2 in terms of fatty acid to lysophospholipid.

[Claim 3] The method described in claim 1 and claim 2, wherein the lysophospholipid is a lysophospholipid derived from plant seeds.

[Claim 4] The method described in claim 3, wherein the lysophospholipid is soy lysophospholipid.

[Claim 5] The method described in claim 1 through claim 4, wherein the fatty acids are one or more fatty acids selected from among those unsaturated fatty acids with 16 or more carbon atoms or from among those saturated fatty acids with 8 to 14 carbon atoms.

[Claim 6] A method for controlling plant blight, wherein a mixture of lysophospholipids and fatty acids is the product of phospholipase A-2 from plant seed-derived phospholipids, and wherein an aqueous solution of this mixture is applied to plants.

[Claim 7] The method described in claim 6, wherein the mixture of lysophospholipids and fatty acids is the product of phospholipase A-2 from soy phospholipids.

[Claim 8] A method for controlling plant blight, wherein a mixture of lysophospholipids and fatty acids also contains plant oils, and wherein an aqueous solution of this mixture is applied to plants.

[Claim 9] The method described in claim 8, wherein the mixture of lysophospholipids and fatty acids containing plant oils is the product of phospholipase A-2 from soy phospholipids.

[Detailed Description of the Present Invention]

[0001]

[Industrial Field of Application] The present invention relates to a method for controlling blights occurring in agricultural products, garden products and garden trees.

[0002]

[Prior Art] Plants cultivated artificially such as crops are affected by more blights and pests than other plants such as trees. Agrochemicals are often used to control these blights and pests. The control of plant blights, which began long ago with the use of Bordeaux mixtures, now commonly takes the form of agrochemicals such as pesticides and medicines (for both prevention and treatment). However, these cellular toxins and metabolic toxins sometimes adversely affect the plants and humans in addition to the pathogens. As organic farming methods have made chemical-free or reduced-chemical farming more popular, the use of agrochemicals is now avoided. However, it is very difficult to prevent blights using organic farming methods and chemical-free cultivation. The chemical-free cultivation of plants in the hot, humid environment of greenhouses has been especially difficult. As a result, a great deal of effort has been directed towards solving this problem in the field of organic cultivation. To this effect, natural materials such as pyroligneous acid are increasingly used for agricultural purposes as soil disinfectants, additives to manure, growth promoters, and insect repellents (see Japanese Examined Patent Application Publication [Kokoku] No. 63-24964, Sanrinshi [Forestry Journal], 1978, p. 28, and Mokuzaigaku Zasshi [Wood Science Journal], Vol. 35, p. 564 and p. 1021).

[0003] The present inventors discovered that (1) a mixture of pyroligneous acid and (2) a mixture of pyroligneous acid, a lysophospholipid and a fatty acid has a multiplying effect on plant activation and insect repellent, and a patent application was filed for these compositions. It was observed that these compositions also had a controlling effect on plant blights, but that (1) was very insufficient and (2) was effective but that the presence of pyroligneous acid was not necessary with respect to plant blight.

[0004]

[Problem Solved by the Invention] A non-toxic, stable blight controlling agent effective on blights caused by fungi is needed for organic greenhouse cultivation, house garden cultivation, fruit trees, garden trees and lawns. However, these means are limited to such things as plant breeding for resistance to blight, graft cutting, and improved cultivation methods. The purpose of the present invention is to control plant blights, especially plant blights caused by fungi, using completely natural substances.

[0005]

[Means of Solving the Problem and Operation] The present inventors with the intention of controlling plant blights using non-toxic, safe means discovered that blights, especially blights caused by fungi, can be controlled by spraying plants with a mixture of lysophospholipids, unsaturated fatty acids/and or in a aqueous micelle solution or aqueous dispersion solution. The present invention is a product of this discovery. In other words, the present invention is a method for controlling plant blight, wherein an aqueous solution comprising a lysophospholipid and fatty acid mixture is applied to plants.

[0006] First, the lysophospholipid to fatty acid mole ratio basic to the present invention is 0.5-2 in terms of fatty acid to lysophospholipid. The lysophospholipid can be an animal lysophospholipid from egg yolks, a plant lysophospholipid from soy, or any other lysophospholipid. However, lysophospholipid derived from plant seeds such as soy lysophospholipid are ideal because more than 50 wt% of the phospholipids are lysophospholipids. Next, the fatty acids used in the present invention can be one or more fatty acids selected from among those unsaturated fatty acids with 16 or more carbon atoms or from among those middle-chain fatty acids with 8 to 14 carbon atoms. For example, a mixture of fatty acids from soybean oil and palm oil can be used.

[0007] Second, after processing the phospholipids with phospholipase A-2, the mixture can be used after the reaction without separating out and removing some or all of the fatty acids. Other fatty acids can then be added for special uses. After sufficient enzymatic reaction, the amount of lysophospholipids contains in the mixture is high. If the fatty acid mole ratio does not exceed 1, a clear or nearly clear aqueous micelle solution or aqueous dispersion solution can be obtained.

[0008] Third, after processing a phospholipid paste containing oils and fats (ordinary soy phospholipids used in food products) with phospholipase A-2, it can be used without separating out some or all of the fatty acids, oils and fats. The mixture can be added to water, heated and stirred to obtain a clear micelle solution or aqueous dispersion solution containing a fine oil and fat emulsion. These are ideal for controlling plant blights in the method of the present invention.

[0009] The lysophospholipids used in the present invention are monoacylated phospholipids obtained by deacylating phospholipids from egg yolks or from plants such as soy, corn or rapeseed using phospholipase A-2. The phospholipase A-2 is usually derived from the pancreas, but phospholipase A derived from microorganisms (A-1 and A-2) can also be used. Gum is a byproduct of soy oil and rapeseed oil production, so a raw phospholipid containing an oil component or a soy lysophospholipid obtained from a raw soy phospholipid paste is inexpensive and ideal for use as a material in the present invention.

[0010] The products of phospholipase and phospholipase (A-1 and A-2) include lysophosphatidylcholine, lysophosphatidylethanolamine, monoacylglycerophospholipids from lysophosphatidic acid and fatty acids. The lysophospholipids refined from these mixtures using acetone or ethylacetate are ideal for use as the material in the present invention. Lysophospholipids can also be obtained through chemical synthesis.

[0011] It is impossible to convert all of the phospholipids into lysophospholipids using enzymatic treatments and some acylated phospholipids remain. However, mixtures in which 50 wt% or more of the phospholipids are lysophospholipids can be used as materials suitable for the present invention. A higher amount of lysophospholipids among the phospholipids is better, but an amount greater than 80 wt% has no significantly greater effect. After the lysophospholipids, e.g., raw soy phospholipids, have been processed with phospholipase A-2, the water can be removed and the oil content and fatty acids extracted using a solvent such as acetone or ethylacetate. In either case, the weight of the lysophospholipids is calculated in absolute terms.

[0012] The unsaturated fatty acids with 16 or more carbon atoms used in the present invention can be linoleic acid, linolenic acid, oleic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid or mixtures of these acids. A small amount of a saturated fatty acid such as palmitic acid or stearic acid can be added. Fatty acids derived from plants such as soy are ideal. The middle-chain fatty acids that can be used in the present invention include caprylic acid, lauric acid, myristic acid or mixtures of these acids. A small amount of another fatty acid can be added. Fatty acids such as those from palm oil or palm kernel oil are ideal.

[0013] In the present invention, with the molecular weight of the lysophospholipids set at 500, the mole ratio to fatty acids (fatty acid/lysophospholipid) is first established for the mixture between 0.5 and 2 in absolute terms. If a fatty acid mixture is used, the average molecular weight is used. An aqueous micelle solution or dispersion solution is prepared for the mixture using an appropriate solvent such as an ethanol/hexane solution. After heating the solution and performing phase-dissolution, the solvent is removed, and the remaining mixture is dissolved under heat in water. If the fatty acids have middle chains and relatively low in quantity in absolute terms with respect to the lysophospholipids, then fatty acids are added directly to an aqueous micelle solution of lysophospholipids under heat and the mixture is stirred until they have dissolved. If the mole ratio is under 0.5, the controlling effect on plant blight is insufficient. If the ratio is above 1, it may still be effective. However, if the ratio is above 1 in the case of unsaturated fatty acids, it is difficult to extract the fatty acids from the lysophospholipid/fatty acid mixture micelle. In the case of capric acid with 10 carbon atoms, the fatty acids will readily precipitate out at a mole ratio above 2.

[0014] When an aqueous micelle solution or aqueous dispersant of the mixture is applied to plants, the concentration should range between 0.2 wt% and 0.005 wt% in terms of the total weight of the lysophospholipids and fatty acids. (Hereinafter, the concentration of the solution of the present invention is indicated in terms of the total weight of the lysophospholipids and fatty acids.) Usually, the solution is sprayed at a concentration between 0.1 wt% and 0.01 wt% on plants before or after the observation of blight. The entire plant is sprayed above ground and on both sides of the leaves. The pH of the solution is usually weakly acidic at 5-6. The solution concentration and spraying frequency depend on the type of plant (grass or tree), whether the plant is embryonic or a seedling, its growth and development, and whether or not blight has been observed.

[0015] After germination of dicotyledonous plants, the solution is applied once there are two or three leaves and then once every 10 or 20 days. In the case of vegetables, a 0.2 wt% solution should be applied to the furrowed soil before the seedlings are planted. In the case of rosaceous fruit trees, they are to be sprayed in early spring before and after budding and then periodically thereafter. If the solution is sprayed on immediately after blight is discovered, it can halt further spread of the disease. The solution of the present invention can be used to effectively control blights caused by fungi that are common among vegetables, fruits and garden plants cultivated in greenhouses such as powdery mildew, bitter rot, green sickness and fusarium wilt.

[0016] The present inventors are aware of mixtures of lysophospholipids, unsaturated fatty acids and/or middle-chain fatty acids with superior surface activation. Unlike highly stable surfactants, these are able to lower surface tension while also providing strong permeability and high leaf surface diffusion (see Japanese Unexamined Patent Application Publication [Kokai] No. 2-203928 and the Journal of the American Oil Chemists' Society, Vol. 67, No. 12, p. 1008 (1990)). However, the superior plant blight controlling effect of these mixtures in aqueous micelle solutions and aqueous dispersants is completely unknown.

[0017] The present inventors are aware of the bacteriolytic effect of lysophosphatidylcholine on Pseudomonas aeruginosa and Streptococcus (M. Rahab et al., Inflammation Journal, Vol. 3, p. 365) and the bacteriolytic effect of different lysophospholipids on microbes of the genus Bacillus (Tsuchida and Fujita, 1991 Presentation to the Japan Society of Fermentation Technology). However, the effect of aqueous micelle solutions of lysophospholipids alone on plant blights is very inadequate.

[0018] The discovery of the significant plant blight controlling effect of fatty acids and lysophospholipids, which are the partial breakdown products of the phospholipid enzymes constituting biomembranes, is truly astonishing. Phospholipase A-2 is a ubiquitous enzyme in cell membranes and organelle membranes, and the activity of this enzyme produces lysophospholipid/fatty acid mixtures locally. Therefore, these mixtures are believed to be harmless to living organisms.

[0019] The reason for this effect is unclear. However, M.J. Bucovach discovered that the structure of a leaf can be penetrated via the stomata if the surface tension of a solution is under 30 mN/m (Plant Physiology Journal, Vol. 49, p. 813 (1972) and American Journal of Botany, Vol. 61, p. 100 (1974)). The surface tension of lysophospholipid/fatty acid aqueous solutions of the present invention up to the micelle concentration limit and at an equal mole concentration of soy lysophospholipid to fatty

acid is 28-29 mN/m in the case of polyhydric unsaturated fatty acids, 25-27 mN/m in the case of middle-chain fatty acids, and 25-28 mN/m in the case of the addition of a small amount of soy oil. Clearly, these aqueous solutions are easily able to penetrate the structure of a leaf via the stomata. Once these solutions have been applied to the surface of a leaf, this effect accelerates as the water component evaporates and the concentration of the mixture increases.

[0020] According to the most recent research by the present inventors, soy lysophospholipids and mixtures of soy lysophospholipids and unsaturated fatty acids or middle-chain fatty acids are bondable to aqueous proteins at room temperature. It is possible therefore that abnormalities occur in the cell membranes of relatively weak fungi and membrane-bonded enzymatic proteins. Lysophospholipids themselves are highly permeable and have even better permeability, moistening and spreadability effects when lysophospholipids are combined with fatty acids. Mixtures of lysophospholipids and polyhydric unsaturated fatty acids have the permeability, moistening and spreadability effects of an industrial surfactant such as polyoxyethylene nonylphenolether even though the actual surfactant effect is different.

[0021] In the embodiments of the present invention, a lysophospholipid/fatty acid mixture or lysophospholipid/fatty acid/oil mixture is applied to plants as an aqueous micelle solution or aqueous dispersion solution. However, other application methods can be used. Usually, the entire plant is sprayed above ground including the leaves and stems. The aqueous micelle solutions and dispersion solutions can be prepared as concentrated and diluted during use, but these forms require preservatives for storage. Other components can be added to the aqueous solution mixtures of the present invention so long as the effects of the present invention remain unaffected. The use of the aqueous solution mixtures of the present invention in tandem with agrochemicals is possible, but a diminished agrochemical effect should be expected.

[0022]

[Working Examples] The following is an explanation with reference to working examples of how the mixtures of the present invention are prepared and how the effects of the present invention are verified. The present invention is by no means restricted to these working examples. In the following explanation, all percentages refer to weight percentages and all parts refer to parts per weight.

[0023] The following is an explanation of the materials used in the working examples of the present invention.

O Lysophospholipid Preparation: An effective method for preparing lysophospholipids is the improved method disclosed in Japanese Unexamined Patent Application Publication [Kokai] No. 64-16595. In this method, 200 g of tap water containing 2 g of pancreatic phospholipase A-2 (Novonordisk Lysidase 10-N) is added to 1 kg of heated oily soy lipidase (Ajinomoto) and stirred to create a W/O emulsion. After displacing the air inside the sealed container with nitrogen gas, the container is allowed to stand for 40 hours at 60°C until the reaction is complete. The oily layer is then separated from the supernatant and removed using centrifugation. Next, 2 kg of acetone is added to the mixture of precipitated reaction product containing the water component and the oil component. The precipitate is separated out using centrifugation, the lysophospholipids undissolved in the acetone are extracted and the solvent is removed to obtain soy

lysophospholipids. The analyzed product contains 80% lysophospholipids. These soy lysophospholipids are referred to as SLP80.

[0024] O Lysophospholipid/Fatty Acid/Oil Mixture Preparation: The reaction product mixture including the water component and oil component obtained using the method described in the previous paragraph contains 30% lysophospholipid and 16% fatty acid, which is primarily linoleic acid. This mixture is referred to as OLF30/16. This reaction product is centrifuged to remove some of the oil layer (oil component and fatty acids) from the supernatant. This contains 34% lysophospholipid and 15% fatty acid and is referred to as OLF34/15.

[0025] O Soy Lysophospholipids: Basis LP-20E (Nisshin Oil Mills Co., Ltd.) was analyzed and found to contain 71% soy lysophospholipid. This product is referred to below as SLP71.

[0026] O Fatty Acids: All of the fatty acids used here were manufactured by NOF Corporation. These include Extralinoleic 90 (90% linoleic acid), Extralinolenic 90 (90% linolenic acid), NAA-122 (99% lauric acid), NAA-102 (99% capric acid) and A-1240 (palm fatty acid).

[0027] Preparation of the Mixture in the Present Invention

Here, 63 parts SLP80 and 28 parts Extralinoleic 90 are phase-dissolved in 500 parts mixed solution such as ethanol and hexane, and the solvent is removed to obtain 18:2/LP80. This is an equal mole mixture of soy lysophospholipid and linoleic acid. Similarly, mixtures are prepared with 28 parts Extralinolenic, 20 parts NAA-122, 17 parts NAA-102 or 21 parts A-1240 added to 63 parts SLP80 in order to obtain 18:3/LP80, 12/LP80, 10/LP80 and CO/LP80, respectively. All of these are equal mole mixtures of soy lysophospholipid and various fatty acids.

[0028] A mixture of 63 parts SLP80 and 14 parts NAA-122 as well as a mixture of 63 parts SLP80 and 30 parts NAA-122 are prepared similarly in the manner described above to obtain 12/LP0.7 and 12/LP1.5, respectively. Also, 28 parts Extralinoleic 90 and 20 parts NAA-122 are processed in the same manner as above in 70 parts SLP71 to obtain mixtures. These are 18:2/LP61 and 12/LP71, respectively.

[0029] Furthermore, 5 parts A-1240 is added to 95 parts OLF34/15, heated to approximately 70°C, and mixed thoroughly. The mixture is OLF/CO/52.

[0030] Preparation of the Aqueous Solution in the Present Invention

The mixtures obtained using the methods described above were heated and dissolved in tap water, and then applied to plants. The concentrations in the following working examples were calculated using the total values for the soy lysophospholipids and fatty acids.

[0031] Working Example 1

In Working Example 1, a total of six variations were tested on strawberries, namely, solutions of 18:2/LP80, 18:3/LP80, 18:2/LP71 and OLF34/15 as well as a SLP80 solution serving as the control. The strawberries (nyoho-variety strawberries) were then

planted in six 8 m^2 plots inside a greenhouse for greenhouse cultivation. After ordinary fertilization, the disease-free seedlings were planted in beds at the end of September. The planting method was flat-furrow four-row planting with a density of eight disease-free seedlings per square meter or 64 seedlings per plot.

[0032] The day before planting, 2 l/m^2 of the various 0.2% aqueous solutions of the present invention and the SLP80 aqueous solution in the comparative example were sprinkled on the test plots (water was used on the control plot). Temperature control was begun in the middle of October (30°C for the duration of the growing season during daylight and about 25°C thereafter with the temperature at 12-7°C at night). Subsequently, mulch and lining curtains were applied, weeding was performed, and bees were introduced. The harvest began at the end of December and cultivation was ended at the beginning of April. In the meantime, spraying with the lysophospholipid and fatty acid solution mixtures was begin about two weeks after planting. The spraying of 0.03% solution was then performed every 10 days until both sides of the leaves were wet. Initially, this was 0.21 per 8 m^3, but this increased to 1-1.21 after growth. While growing the leaves and fruit without the application of any agrochemicals, they were observed for powdery mildew, bitter rot, green sickness and fusarium wilt. The number of plants stricken with these blights were recorded. It is clear from the results shown in Table 1 that the control group was stricken severely with blight but that the treated plants were much less stricken with blight. In other words, the present invention has a blight controlling effect.

[0033]

[Table 1]

Number of Diseased Fruit (In Group of 64 Strawberries) in Working Example 1 and Working Example 2

Solution / Mixture	Powdery Mildew	Bitter Rot	Green Sickness	Fusarium Wilt	Total
18:2/LP80	6	1	2	0	9
18:3/LP80	4	2	1	1	8
18:2/LP71	7	2	2	1	12
OLF34/15	5	1	2	0	8
SLP80	27	6	8	2	43
Control Group	36	8	12	5	61
12/LP80	5	0	3	0	8
10/LP80	4	1	3	0	8
12/LPO.7	11	1	2	1	15
12/LP1.5	5	1	2	1	9
12/LP71	6	2	2	0	10
Control Group	31	7	11	3	52

[0034] Working Example 2

In Working Example 2, as in Working Example 1, a total of six variations were tested on strawberries (nyoho-variety strawberries), namely, solutions of 12/LP80, 10/LP80, 12/LP0.7, 12/LP1.5, and OLF30/16 as well as the control. It is clear from the results shown in Table 1 that the control group was stricken severely with blight but that the treated plants were much less stricken with blight. In other words, the present invention has a blight controlling effect.

[0035] Working Example 3

In Working Example 3, a total of six variations were tested on cucumber, namely, solutions of 18:2/LP80, CO/LP80, OLF30/16, OLF/CO/52 and 12/LP71 as well as the control. The cucumbers were planted in six 2.5 x 4 = 10 m^2 plots inside a greenhouse for intensive cultivation. The salt removal, steam sterilization and fertilization were performed, the soil was mulched, and 20 grafted disease-free seedlings were planted in beds with two rows at the end of December (cucumber variety: Sharp 1, Saitama Stock Seed Cultivation Association). After mulching, 4 l/m^2 of the various aqueous solutions of the present invention were sprinkled at 0.2% on the test plots (water was used on the control plot). Next, tunnels were built for temperature control. Twenty days later, after observing how well the seedlings had taken root, the vines were tied and the temperature was maintained at approximately 25°C during the day and approximately 12°C at night.

[0036] Next, 0.03% lysophospholipid and fatty acid solution mixtures of the present invention were sprayed on the cucumbers until both sides of the leaves were wet 20 days after planting and every 10 days thereafter. Initially, this was 0.41 per 10 m^3, but this increased to 1.2-1.51 after growth. The harvest began at the middle of February and cultivation was completed at the end of May. Irrigation and fertilization were performed in the usual manner during cultivation, but no agrochemicals were applied. All the while, the cucumbers were observed for downy mildew, botrytis rot and powdery mildew. The number of plants stricken with these blights were recorded. It is clear from the results shown in Table 2 that the control group was stricken severely with blight but that the treated plants were much less stricken with blight. In other words, the present invention has a blight controlling effect.

[0037]

[Table 2]

Number of Diseased Vegetables (In Group of 20 Cucumbers) in Working Example 3

Solution / Mixture	Downy Mildew	Botrytis Rot	Powdery Mildew	Total
18:2/LP80	1	1	4	6
CO/LP80	1	1	5	7
OLF30/16	0	2	4	6
OLF/CO/52	0	1	4	5
12/LP71	1	1	5	7
Control Group	6	6	15	27

[0038]

[Effect of the Invention] The effect of the present invention is the discovery of a novel blight control method for plants using organic materials and the partial breakdown products of highly stable lysophospholipids. This has provided a new way of cultivating vegetables and garden plants without the use of agrochemicals.